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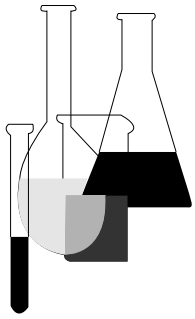
Health Effects Test Guidelines

OPPTS 870.6855 Neurophysiology: Sensory Evoked Potentials



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 870.6855 Neurophysiology: sensory evoked potentials.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** This guideline was developed jointly between the Office of Pesticide Programs and the Office of Pollution Prevention and Toxics in cooperation with the Office of Research and Development of the Environmental Protection Agency. The source material used in developing this harmonized guideline is 40 CFR 798.6855 Neurophysiology: sensory evoked potentials.

(b) **Purpose.** The techniques in this guideline are designed to detect and characterize changes in the sensory aspects of nervous system function that result from exposure to chemical substances. The techniques involve neurophysiological measurements from adult animals and are sensitive to changes in the function of auditory, somatosensory (body sensation) and visual sensory systems. These procedures can be used in two ways:

(1) To detect sensory dysfunction produced by compounds in the absence of relevant information.

(2) When there are reasons to expect that particular sensory functions are specifically sensitive to the test compound. The procedures employed during a particular study will be selected on a case-by-case basis depending on information available at the time of the study design, signs of toxicity observed during the study, and/or the purpose of the study. It will be the responsibility of those submitting to justify the selection of a specific test from the categories of electrophysiological tests available. The tests are adaptable so that they may be used in sum or in part, and either alone or in conjunction with other tests including: A functional observational battery, motor activity, neuropathology, and general toxicity studies. These studies may involve acute, subchronic, or chronic exposures.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Neurotoxicity is any adverse effect on the structure or function of the nervous system related to exposure to a chemical substance.

Toxic effect is any adverse change in structure or function of an experimental animal as a result of exposure to a chemical substance.

(d) **Principle of the test method.** The test substance is administered to several groups of experimental animals, each group receiving a different dose level. Electrodes for recording brain electrical activity are temporarily

or permanently affixed to the test animals. After electrodes are in place and, if appropriate surgical recovery, stimuli for the visual, auditory or somatosensory sensory systems are presented to the test animals, and the resulting brain electrical potentials are recorded. The electrical potentials recorded from animals treated with the test compound are compared to those recorded from control animals. The results are interpreted regarding the extent to which treatment with the test compound altered the normal function of the sensory systems tested.

(e) **Test procedure**—(1) **Animal selection**—(i) **Species and strain.** Testing should generally be performed in the laboratory rat, preferably a pigmented strain. Albino strains of animals have abnormalities of the visual and auditory systems (see paragraphs (g)(4), (g)(5), and (g)(14) of this guideline), including: The absence of pigment from the retinal pigment epithelial layer, high incidence of spontaneous retinal pathologies, problems of photogenic retinopathy (under paragraph (g)(8) of this guideline), abnormal pattern visual evoked potentials (under paragraph (g)(1) of this guideline), lack of normal pigment in the stria vascularis of the cochlea (see paragraph (g)(18) of this guideline) and differential susceptibility to ototoxic noise and drugs from pigmented strains (under paragraphs (g)(2) and (g)(3) of this guideline). However, it is recognized that under some circumstances, use of another species or an albino strain may be justified. If another species or an albino strain is used, the user must submit justification.

(ii) **Age.** Animals should be young adults (42–120 days of age, for rats) at the start of exposure. Implantation of chronic electrodes in rats younger than 60 days of age is not advised.

(iii) **Sex.** (A) In order to reduce the number of animals used, only one sex is required. Male rats may be preferred because there is more existing data on them. If, on the other hand, females are known or expected to be more sensitive to the test agent, they may be used. The user should provide justification for the sex selected.

(B) If females are used, they should be nulliparous and nonpregnant.

(2) **Number of animals.** A final sample size of at least 10 animals should be used in each dose and control group. The number of animals to be used should be based on appropriate statistical methods and an allowance for attrition due to anticipated problems such as loss of electrode preparations, etc. Note that the rate of attrition should be estimated based on the experience of the laboratory performing the testing. If interim neurophysiological evaluations are planned in long-term dosing studies, it may be advisable to include an additional number of animals sufficient for the interim studies. Animals should be randomly assigned to treatment and control groups. If groups are not randomly assigned, justification must be provided.

(3) **Control groups.** (i) A concurrent (‘‘sham’’ exposure) vehicle control group is required. Subjects should be treated in the same way as an exposure group except that administration of the test substance is omitted. If the vehicle used has known or potential neuroactive properties, both untreated and vehicle-control groups are required.

(ii) Positive control groups exhibiting functional changes in the sensory systems to be tested are required in order to demonstrate the capability of the laboratory performing the testing to conduct the procedures. Separate setups for each sensory system are acceptable, but not necessary. In addition, for each sensory modality in vehicle or untreated control group data, it should be demonstrated that the mean of an amplitude-sensitive dependent measure increases monotonically as a function of stimulus intensity as defined in paragraph (e)(7)(v)(D) of this guideline. Historical data may be used if the essential aspects of the experimental procedure remain the same. Periodic updating of positive control data is recommended. New positive control data should also be collected when personnel or some other critical element in the testing laboratory has changed.

(4) **Dose levels and dose selection.** (i) At least three dose levels should be used in addition to the vehicle control group. Ideally, the data should be sufficient to produce a dose-effect curve. We encourage the use of equally spaced doses on a logarithmic scale, and a rationale for dose selection that will enable detection of dose-effect relations to the highest degree possible. For acute studies, dose selection may be made relative to the establishment of a benchmark dose (BD). That is, doses may be specified as successive fractions, e.g. 0.5, 0.25 of the BD. The BD itself may be estimated as the highest nonlethal dose as determined in a preliminary range-finding study. A variety of test methodologies may be used to determine a BD, and the method chosen may influence subsequent dose selection. The goal is to use a dose level that is sufficient to be judged a limit dose, or clearly toxic. Alternatively, the BD may be specified as a dose of the test compound producing clear neurotoxic effects in previous studies.

(ii) **Acute studies.** The high dose need not be greater than 2 g/kg. Otherwise, the high dose should result in significant neurotoxic effects or other clearly toxic effects, but not result in an incidence of fatalities that would preclude a meaningful evaluation of the data. This dose may be estimated by a BD procedure as described above, with the middle and low dose levels chosen as fractions of the BD. The lowest dose should produce minimal effects or, alternatively, no effects.

(iii) **Subchronic and chronic studies.** The high dose need not be greater than 1 g/kg/day. Otherwise, the high dose level should result in significant neurotoxic effects or other clearly toxic effects, but not produce an incidence of fatalities that would preclude a meaningful evaluation of

the data. The middle and low dose should be fractions of the high dose. The low dose should produce minimal effects, or alternatively, no effects.

(5) **Route of exposure.** Selection of route may be based on several criteria: The most likely route of human exposure, the greater likelihood of observing effects, the practical difficulties, the likelihood of producing nonspecific effects, and existing data regarding the test compound. Because more than one route of exposure may be important for many materials, these criteria may conflict with one another. The route that best meets these criteria should be selected.

(6) **Combined protocol.** The tests described herein may be combined with any other toxicity study, as long as none of the requirements of either is violated by the combination.

(7) **Study conduct**—(i) **Preparation of animals for recording.** (A) For electrophysiological recording it usually will be necessary to implant animals with chronic in-dwelling electrodes using stereotaxic surgical procedures. In some circumstances, acute attachment of temporary electrodes may be acceptable if criteria for humane treatment of animals, for recording without undue anesthesia, and for data acceptability detailed below can be met. Chronic implantation of electrodes will require surgical anesthesia and surgical techniques appropriate for the species as outlined in current laboratory animal care guidelines under paragraph (g)(17) of this guideline. Standard animal surgical practices should be followed as outlined in a number of standard references (e.g. paragraph (g)(11) of this guideline). Once anesthetized, animals are usually placed in a stereotaxic device in order to position the head firmly. The stereotaxic device should be designed to prevent trauma to the tympanic membranes and, for auditory studies, tympanic membranes should be examined after removal from the stereotaxic device.

(B) Care should be taken during surgery to prevent drying of the cornea through means such as regular application of fluids such as mineral oil, saline, or artificial tear solution. Once the animal is positioned in the stereotaxic device and the implantation site is prepared, the electrodes should be positioned with reference to standard skull markings and/or to published brain atlas coordinates (see paragraph (g)(12) or (g)(13) of this guideline).

(C) For recording potentials which are generated in sensory cortex, the recording or active electrodes are to be positioned as close as possible to the brain sites generating the response. For ‘‘far-field’’ potentials such as the brainstem auditory evoked potential, which are conducted through cranial tissues from sites relatively distant to the recording electrodes, the location of the recording electrodes should be such as to provide good resolution of the major waveform components, but need not be as close as possible to the generator sites. The site of the reference electrode for

differential recordings should be indifferent with respect to stimulus-evoked electrical activity to the extent possible. A ground electrode should also be included. The electrodes should be made of a material that is not toxic to neural tissue. The electrodes should be made of a nonpolarizable material, such as silver-silver chloride, if potentials of a frequency less than approximately 1 Hz are to be reported. However, extra caution should be exercised in this case to avoid toxic effects of such electrodes. Electrodes should be described as to composition, size, shape, and position.

(D) Wound sites should be treated and closed so as to prevent infections and to protect the integrity of the electrodes. Following surgery, electrode impedance should be measured in order to verify that electrode connections are intact and functional. Following surgery, animals should be given sufficient time to recover from the anesthetic and surgical trauma prior to testing; ordinarily a period of one week is recommended. Prior to testing, the wound site should be inspected for signs of infection or inflammation, and any animal showing such signs should be removed from the experiment.

(E) For acute studies, it is necessary to perform surgery prior to administering the test compound. In repeated dosing, it may be necessary to implant the electrodes during the course of treatment with the test compound due to the limited time which electrode preparations may be expected to remain intact. It may be advisable to prevent exposure to the test compound on the day of surgery due to possible interactions of the effects of the test compound and the anesthetic agent. The time between surgical implantation of electrodes and testing should be equal for all animals or, if unequal, balanced across the treatment groups. Animals losing electrode preparations between time of surgery and testing should be removed from the study and not submitted to another surgery. At termination of the study, postmortem examination of each animal should be used to determine if the electrodes were incorrectly positioned, or if the presence of epidural electrodes damaged the underlying neural tissue. If either of these two conditions is found, the data from the involved animal should be discarded.

(ii) **Testing environment.** (A) Electrophysiological testing should be conducted in a chamber or room which is isolated from extraneous light and noise and controlled for temperature. Background noise, light, and room temperature should be reported. Where possible, testing should be conducted without the use of restraint or anesthetics. The use of restraint may be necessary when a specific orientation to, or distance from, the stimulation equipment is required, or when movement of the animal would interfere with recording.

(B) For auditory testing of unrestrained, unanesthetized animals, acoustic stimuli should be of equal sound pressure level wherever the animals' ears may be placed during data acquisition. The animal enclosure

should be acoustically transparent to sound in the frequency band of the stimulus spectrum. The use of anesthetic is permissible when considerations of animal discomfort prohibit testing awake animals, and when it can be reasonably expected that the effects of the test agent would not be substantially different under anesthesia. If recording is to be performed on anesthetized animals, body temperature should be maintained within a normal range during testing. In addition, if recording from anesthetized animals, the power spectrum of the spontaneous electroencephalogram (EEG) should be measured in order to control depth of anesthesia. Position of the stimulation device or devices relative to the appropriate sensory organs should be specified.

(iii) **Electrophysiological recording.** (A) Electrophysiological recording procedures should follow generally accepted practice such as is found in standard reference texts (under paragraph (g)(9) or (g)(16) of this guideline). Typically, it is appropriate to differentially record between active and reference electrodes using an amplifier with high common mode rejection and high input impedance relative to that of the electrodes.

(B) Electrical shielding and grounding should be used to eliminate activity in the electrophysiological recording at the frequency of the power lines reflecting inductive noise. Electrophysiological amplifiers and filter bandpass settings must be appropriate to the signal being measured. For example, ac-coupling is appropriate for most applications, but dc-coupling should be used if steady potentials are to be measured. Analog electrical activity is typically digitized using an analog-to-digital converter operating at a rate at least twice, preferable higher, the highest frequency passing the input filters. Analog or digital filtering of data is appropriate to improve signal-to-noise ratios. If using analog filtering, the decay functions of the high and low bands of the filters should be specified, and filtering parameters should be selected to avoid amplitude reductions or phase shifts in the frequency bands of the signal to be measured.

(iv) **Signal averaging.** (A) Signal averaging of the input data synchronized with the stimulation is appropriate to increase the signal-to-noise ratio. The number of trials averaged should be sufficient to yield reliable data, and a waveform in control animals for which the maximum amplitude measure to be reported at the minimum intensity stimulus is at least 50 percent greater in amplitude than the recording noise level. The duration of the sampling epoch should be sufficient to encompass all major components of transient evoked potentials. Automated artifact rejection routines may be employed to reject occasional spurious data provided that no greater than 50 percent of the original trials are rejected for any given waveform, and all final averaged waveforms for a given stimulus condition are based on an equal number of trials.

(B) The data for cases in which greater than 50 percent of the trials are rejected due to artifacts should be discarded, and the recording condi-

tions improved, if possible, to allow more suitable data collection. The recording noise level should be determined in order to illustrate the signal-to-noise ratio. Acceptable methods to do so include: examining a portion of the data preceding the presentation of the stimulus, but following the last of the evoked activity from the previous stimulus in transient evoked potentials; averaging an equal number of trials as the recording session but without presentation of the eliciting stimulus (for visual stimuli this should be accomplished by temporarily blocking the test subjects view of the stimulus with an opaque material); averaging alternate trials of inverted polarity during the standard recording session, or reaveraging the original raw data from the standard recording session over a similar number of trials in a manner nonsynchronous with the eliciting stimulus. The voltage gain and temporal response properties of the electrophysiological recording equipment should be calibrated for each experiment, or more frequently as needed. The same recording conditions should be used on all animals tested.

(v) **Stimulation.** The selection of stimulation parameters will depend upon the specific goals of the study. Tests should include measures of visual, auditory and somatosensory systems, unless there are reasons to limit testing to particular aspects of sensory function. The testing of multiple sensory systems in the same animal has been demonstrated (see paragraphs (g)(10) and (g)(15) of this guideline). The order of presentation of the different tests to individual subjects be either random, balanced across treatment groups, or fixed. If the test order is fixed, justification for the test order should be provided.

(A) **Visual stimuli.** Visual testing should include separate tests involving light flashes and patterned stimuli. Visual pattern testing should employ stimuli with a sinusoidal spatial luminance profile, and should include a range of pattern sizes which encompass the low, middle and high spatial frequency ranges of the contrast sensitivity function of the test species. Techniques for recording visual evoked potentials using both flashed (under paragraph (g)(6) of this guideline) and patterned stimuli (under paragraph (g)(1) of this guideline) are available for laboratory rats.

(B) **Auditory stimuli.** Auditory testing should include a stimulus of broadband frequency characteristics, such as a click. In addition, pure tone stimuli reflecting the low, middle and high frequency portions of the audiometric function of the test species should be used. Techniques for recording auditory evoked potentials using both click and pure tone stimuli (under paragraph (g)(15) of this guideline) are available for laboratory rats.

(C) **Somatosensory stimuli.** Somatosensory testing should include electrical stimuli delivered to the tail or distal portions of the lower extremities. Techniques for recording somatosensory evoked potentials are available for laboratory rats (under paragraph (g)(15) of this guideline).

(D) **Stimulus levels.** (*1*) For studies designed to detect a change in sensory function produced by a compound for which little is known, it is sufficient to use a single stimulus level for each visual, auditory or somatosensory evoked potential. Justification for the stimulus level selected should be provided. For studies designed to characterize a sensory effect, at least three different levels of flash intensity, pattern contrast, acoustic click stimulus sound pressure level or somatosensory stimulus current are required for each stimulus condition. The stimulus levels should be chosen on the basis of prior experience and/or pilot studies.

(*2*) In control animals, the low level should be near the response threshold, but large enough to produce a response amplitude at least 50 percent greater than the recording noise level. Specification of the high stimulus level varies with stimulus type. For flashed visual stimuli the high stimulus level should either produce a maximal response, or be the maximum output available from a conventional stimulator (e.g. Grass model PS-22 photic stimulator). For patterned visual stimuli the high stimulus level should either produce a maximal response, or be below the highest level of contrast within the linear range of the input-voltage/stimulus-contrast calibration function of the stimulus screen. For somatosensory stimuli the high stimulus level should either produce a maximal response, or be at or below a current which produces minimal reflexive muscle movement or other indications of discomfort. For acoustic stimuli the high stimulus level should be below approximately 80 decibels sound pressure level in order to avoid production of temporary or permanent threshold shifts.

(*3*) For all types of stimuli, the middle stimulus level should produce a response intermediate between high and low stimulus levels. The physical and temporal parameters of stimuli should be calibrated against known standards for each experiment using commonly accepted procedures.

(*4*) Visual stimulus luminance and contrast, or for flashes integrated power, should be calibrated with an appropriate radiometer or photometer against a known standard. Acoustic stimuli should be calibrated for level using equipment which meets standards of the American National Standards Institute (ANSI) for sound level meters. Stimuli should be measured and reported as peak levels, maximum root mean square (max RMS), or ‘‘peak equivalent’’ sound pressure levels. In addition, the polarity of the electrical stimulus and the transduction system for acoustic stimuli should be reported.

(vi) **Measurement.** (A) Dependent measures should be taken from each evoked potential which are sensitive to changes of both amplitude (voltage) and latency (time after stimulus onset) for transient evoked potentials or phase for steady-state evoked potentials. Enough measures should be taken to adequately reflect the shape of the evoked potential in control animals.

(B) A variety of measurement schemes may be acceptable, provided they are specified a priori, do not ignore major portions of the waveform, yield ratio-scale values, and meet the criterion for positive controls specified in paragraph (e)(3)(ii) of this guideline. If the measurement scheme involves inspection of each waveform by an operator and scoring of the waveform, the criteria for scoring should be objective and stated.

(C) The experiment also must be conducted so that the experimental personnel are unaware of the treatment of individual animals at the time of scoring. Measurement schemes that minimize the scoring by personnel of each response are preferred. The same data scoring procedure must be used on all subjects.

(D) Previously collected data demonstrating selective effects of the test compound may be used to restrict the number of test parameters and/or the number of measured endpoints in order to examine more restricted hypotheses. Colonic temperature should be measured at the time of each electrophysiological recording. Body weight should be measured on each test day.

(vii) **Acute.** Testing should be timed to include the estimated time of peak effect.

(viii) **Repeated dosing.** Testing should be conducted after the completion of dosing with the test compound when it can be expected that transient effects of the final treatment have dissipated. Additional testing may be conducted during the course of treatment in order to provide information on the emergence of toxic effects.

(f) **Data reporting and evaluation.** The following should be reported:

(1) **Description of the test methods.** This must include:

(i) Positive control data from the laboratory performing the test which demonstrate the sensitivity of the procedure being used. Historical control data can be critical in the interpretation of study findings. We require submission of such data to facilitate the rapid review of the significance of the observed effects.

(ii) Procedures for calibrating the stimulation and recording equipment and balancing the groups.

(2) **Results.** The following must be arranged by test group (dose level).

(i) In tabular form, data must be provided showing for each animal:

(A) Its identification number.

(B) Body weight for each day tested.

(C) Values of each evoked potential dependent measure

(D) Body temperature of the animal at the time of acquisition of each evoked potential.

(iii) Group summary data should also be reported. Data reporting should include in tabular form measures of central tendency and variability for each combination of stimulus conditions and treatment with the test compound, and the statistical significance level for effects of treatment with the test compound associated with each set of values. The final sample size should be reported along with reasons for excluded or missing data. The noise level should be reported. Graphic presentation of the data, or portions thereof, may also be included. Also, samples of typical individual animal evoked potential waveforms illustrating all stimulus conditions and the noise level, or preferably group mean waveforms of the same, should be included.

(3) **Evaluations of the data**—(i) **Data analysis.** (A) Numerical data analysis should include a measure of central tendency, such as mean, and a measure of variability, such as standard error of the mean, for each stimulus and dose treatment combination, and for each dependent variable.

(B) Statistical analysis should test the null hypothesis of no statistically significant overall effect of treatment with the test compound across stimulus conditions for each type of sensory evoked potential. In addition, statistical tests for interactions between treatment with the test compound and the manipulation of the stimulus parameters should be performed and the results reported. The choice of statistical analysis should consider the experimental design and address the problem of adjustments for multiple statistical analyses.

(ii) **Interpretation.** The report should include an interpretation of the neurotoxicological significance of the findings, and relate the neurophysiological results to those of other neurotoxicological results, and to other data to the extent possible. Guidance for interpretation of sensory evoked potential data is described under paragraph (g)(18) of this guideline.

(g) **References.** The following references should be consulted for additional background information on this guideline.

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